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Valorization of onion solid waste and their flavonols for assessment of cytotoxicity, enzyme inhibitory and antioxidant activities

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ABSTRACT

Onion (*Allium cepa* L.) is rich with flavonols which perceived benefits to human health. Flavonols like quercetin and quercetin glycosides from onion solid waste (OSW) have been extracted and tested against enzymes of clinical importance in Alzheimer's disease and diabetes and be shown to have cytotoxic and antioxidant effects. A simple high-performance liquid chromatography-diode array detector method using a Zorbax Eclipse XDB C18 column was developed to separate quercetin-3, 4′-O-diglucoside, quercetin-4′-O-monoglucoside, and quercetin from OSW. These compounds were identified using infrared, ultra-violet, ^{1}H, and ^{13}C nuclear magnetic resonance spectroscopic techniques. The OSW solvent fractions and flavonols showed significant antioxidant activities using DPPH (1, 1-diphenyl-2-picrylhydrazyl), FRAP (ferric reducing antioxidant power), and ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging assays. The samples exhibited significant in vitro anti-cholinesterase activity with strong antidiabetic effects. OSW extracted with methanol and ethanol showed greater in vitro anti-cholinesterase and hypoglycemic effects than QDG, QMG, and Q possibly due to interactions between multiple compounds and/or complex multivariate interactions with other factors in OSW. In addition, cytotoxicity assays showed that OSW and QDG, QMG, and Q could inhibit the proliferation of selected cancer cell lines. Results indicate that OSW and flavonol glycosides are potential antioxidant, antidiabetic, anticancer, and sedative agents.

1. Introduction

Onion (*Allium cepa* L.) is one of the most commonly cultivated vegetable crops across the world, and its production is increasing every year in response to increasing consumer demand. Simultaneously, huge amounts of onion solid waste (OSW) are produced from different parts and processing of the onions, affecting the environment in various ways. The production and consumption of onions is increasing because of their nutritional, medicinal, and functional properties (Sharma et al., 2016). Processed onions can be dehydrated, canned, or pickled. Dehydrated onions, in the form of flakes or powder, are in high demand in many parts of the world. Moreover, increasing demand for processed onions has led to an increase in the production of OSW (Mitra et al., 2012). OSW includes onion skins generated by industrial peeling, two outer fleshy scales, roots, and the top and bottom of bulbs, as well as undersized, malformed, diseased, or damaged bulbs (Sharma et al., 2016). OSW also includes the outer papery brown, yellow, or red skins with the colored tops and bottoms of onion bulbs. The external and internal scales of onions are white and fleshy with a high amount of phenolics and organosulfur compounds (Khiari et al., 2009). Dietary polyphenolics, which significantly occur in many plant foods, are considered as the most bioactive constituents among the plant-derived compounds in vitro and *in vivo* (Chen et al., 2017). Polyphenolic compounds were consumed and utilized most abundantly in our daily diet as food or medicine. These are mainly classified as flavanols, flavonols, flavones, flavanones, isoflavones and anthocyanins (Chen et al., 2018). These polyphenolics including phenolic acids, flavonoids, and flavonol glycosides from different fruits and vegetables are known for their biological effects, including anticancer, anti-mutagenic, antioxidant, anti-proliferative, and antimicrobial effects (Sharma et al., 2016; Miron et al., 2017). Waste products produced from industrially processed onions consist of a significant amount of dietary and phenolic compounds and are rich in the two types of phytochemicals, including flavonoids and alk(en)yl cysteine sulfoxides, which are beneficial to

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human health (Nile et al., 2017a). Flavonols are potential antioxidants found in a wide range of foods; they are particularly enriched in onions. Sixteen different flavonols, consisting of the aglycones and glycosylated derivatives of quercetin, have been identified in onions (Ko et al., 2015). Onions contains various flavonols, including quercetin, quercetin 3,4′-diglucoside, quercetin 4′-glucoside, quercetin 3-glucoside, isorhamnetin 3,4′-diglucoside, and isorhamnetin 4′-glucoside, which comprise approximately 90% of the onion bulb and inner and outer scales of an onion (Benítez et al., 2011). The health-related effects of onions are associated with one or more bioactive compounds such as flavonols, which show anti-carcinogenic, anti-cholesterol, anti-diabetic, antifungal, anti-inflammatory, antimicrobial, anti-osteoporosis, antioxidant, and hypotensive activities (Lee et al., 2008; Sharma et al., 2016). Quercetin is also known to have pronounced effects on allergies, asthma, arthritis, cancer, coronary heart disease, diabetic complications, gout, neurodegenerative disorders, and osteoporosis (Ko et al., 2016; Nile et al., 2017a; Curti et al., 2017). Most bioactive components in onions have not been found in the scales of the bulb; these components are oxidation products of quercetin and its glucosides. Some of these phenolic compounds have been shown to possess strong antioxidant activity in vitro (Khiari and Makris, 2012; Nile and Park, 2013). Knowledge of the health-promoting potential of polyphenolic phytochemicals and their function as antioxidants has led to numerous investigations to exploit agro-food waste materials (Sharma et al., 2015; Petropoulos et al., 2016), with efforts focused mainly on the extraction of polyphenols with functional properties. Thus, eating onions is reported to have several beneficial effects on health, such as preventing tumors, cancers, and cardiovascular diseases, reducing blood sugar levels, and various other biological effects (Lee et al., 2013; Sharma et al., 2016). Because of this, the possibility of recovering antioxidants like flavonols from OSW as sources for functional characterization and use these flavonols has been the subject of many in vitro studies. However, to the best of our knowledge, there are no specific reports on the valorization of OSW and their flavonols for assessment of acetylcholinesterase (AChE) butyrylcholinesterase (BuChE), α-amylase, and α-glucosidase enzyme inhibitory activity and cytotoxicity in the literature studied previously. So this study aimed to quantify and isolate quercetin and quercetin glycosides from OSW and to determine whether these compounds potentially inhibit acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), α-amylase, and α-glucosidase enzymes which are associated with Alzheimer's disease and diabetes. Further these isolated flavonols were subjected to design and development of lead natural drugs with the useful therapeutic tool for control of activities of these enzymes with possible antioxidant activity.

2. Materials and methods

2.1. Chemicals

All chemicals and solvents used in this study were of high-performance liquid chromatography (HPLC) analytical grade. 2,2-Azinobis (3-ethyl benzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS), butylated hydroxy anisole (BHA), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), β-carotene, linoleic acid, 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), α-amylase, α-glucosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), acetylthiocholine (ATC), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), S-butyrylthiocholine chloride, physostigmine, and galanthamine hydrobromide were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Sodium acetate trihydrate, ferric chloride, trichloroacetic acid, (TCA), 2-thioharbituric acid (TBA), gallic acid and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were purchased from Sigma-Aldrich Co. (Seoul, Republic of Korea). Quercetin (Q), quercetin-4′-O-monoglucoside (QMG), quercetin-3, 4′-O-diglucoside (QDG) were supplied by Sigma-Aldrich Co. (St. Louis MO, USA) (Fig. 1). Methanol and ethyl acetate were obtained from Duksan Chemicals (Seoul, Republic of Korea). The purity of all chemicals used as standards was found to be > 99% by HPLC analysis.

2.2. Onion solid waste (OSW)

OSW was obtained from Mokpo Experimental Station, National Institute of Crop Science, Muan (Republic of Korea) from processed yellow onion (*Allium cepa* L. cv. Sunpower) bulbs. Ten kilograms of OSW consisted of the edible dry outer layers and apical and basal trimmings of onions (Fig. 2). The samples were freeze-dried and milled using an electrical grinder (HR2100, Philips Electronics Korea Ltd, Seoul, Korea) and passed through a 1.0-mm sieve. Immediately after processing, the OSW was transferred to the laboratory and stored at −20 °C until further analysis.

2.3. Extraction of OSW

One hundred grams of OSW was finely ground using a domestic blender (HR2100; Philips Electronics Korea Ltd., Seoul, Republic of Korea) and macerated with 80% aqueous methanol (5 mL per 1 g of OSW) for 48 h at 30 °C. The sample was then stirred using a magnetic stirrer at 20,000 rpm for 60 min and then filtered through Whatman filter paper No. 1. After filtration, the solvent (methanol) was then removed from the filtrate using a rotary evaporator at 60 ± 0.5 °C. This procedure was repeated three times until a consistent product was obtained. The filtrate was adjusted to its final volume in a 100-mL volumetric flask. The obtained OSW extract was suspended in water and successively diluted in different solvents, including 80% aqueous methanol, 80% aqueous ethanol, and ethyl acetate to extract OSW for 48 h at 30 °C to obtain various fractions, each of which was collected for further studies (Nile et al., 2017a).

2.4. Phytochemical characterization

Total phenolic content (TPC) and total flavonoid content (TFC) were determined for each OSW fraction extracted with 80% aqueous methanol, 80% aqueous ethanol, or ethyl acetate (5 mg/mL). The Folin-Ciocalteu method was used to determine TPC (Nile et al., 2017b). TFC was determined using the $AlCl_3$ complexation method (Majid et al., 2016). The absorbance of a sample was measured on a Shimadzu UV-160A spectrophotometer. TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry weight (DW). Total flavonoid content was expressed as milligrams of quercetin equivalent (QE) per gram of dry weight (DW).

2.5. HPLC analysis and identification of quercetin and quercetin glycosides in OSW

An HPLC analysis was conducted using an Agilent 1100 chromatograph (Agilent Technologies; Palo Alto, CA, USA) equipped with a binary pump, auto-sampler, column heater, solvent delivery system, and diode array detector (DAD-370 nm), and Chem-Station data acquisition system. A Zorbax Eclipse XDB C18 column (250 mm × 4.6 mm) with a particle size of 5 μm (Agilent; Santa Clara CA, USA) and protected with a Phenomenex (USA) C18 type guard column was used for the analysis of quercetin-flavonols. Quercetin and quercetin glycosides were separated using a gradient elution with a mobile phase of 0.5% trifluoroacetic acid (TFA) in water and methanol. The gradient program was as follows: 0–10 min, 20% methanol; 10–15 min, 20–80% methanol; 15–22 min, 80–20% methanol. The flow rate was 0.8 mL/min, injected volume was 10 μL, run time of 30 min, column temperature was 25 °C, and wavelength used for monitoring was 360 nm. The chromatographic analysis of each replicate sample was performed in triplicate, and the average peak areas were used in calculations. The Q, QMG, and QDG were identified and verified by comparison with available standards and/or exact mass measurements